



Research Paper

Drought changes yield and organic and mineral composition of grains of four maize genotypes

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ABSTRACT

When drought occurs in the pre-flowering of maize with high intensity and average duration of twelve days, it promotes changes in the centesimal composition of grain and reduces yield grains by up to 60%. Therefore, this work aims to evaluate the changes in yield grain and in organic and mineral composition of grains of four maize genotypes with contrasting characteristics for drought tolerance submitted to severe drought in pre-flowering. For this, under greenhouse conditions, drought-tolerant genotypes (DKB390 and P30F35) and drought-sensitive genotypes (BRS1010 and 2B710) of maize were submitted to two water conditions: field capacity and water deficit. Drought was imposed in pre-flowering and maintained for twelve days. The leaf water potential and at the end of the cycle the components of productivity was evaluated. Dry grains were ground and a sample obtained for analysis of organic and mineral composition. We verified that, water deficit promoted a reduction in the number of grains per row in all genotypes. However, only BRS1010 and 2B710, two drought-sensitive genotypes, had their final grain yield reduced. All genotypes under drought conditions reduced starch content and increased protein levels in their grains. Phosphorus, iron, magnesium, zinc, manganese and copper levels increased in plants under water deficit. On the other hand, calcium content was reduced by drought in all genotypes.

Keywords: Water deficit. *Zea mays*, centesimal composition of grain, starch, protein.

Abbreviations: **FC:** Field capacity; **WD:** Water deficit; **RS:** Reducing sugars; **ADF:** Fiber in acid detergent; **DM:** Dry matter.

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INTRODUCTION

In 2050, the world population is projected to reach approximately 9.6 billion people (Lee et al., 2011). In view of this, it is estimated that cereal production to feed this growing population will have to rise from 2.1 billion tons, currently, to 3 billion tons in 2050 (Food and Agriculture Organization of the United Nations, 2015). This generated major concerns in world food security, since the rate of improvement in yield per area of even the major crops in some areas of the globe is stagnant or even moving in the opposite direction (Long et al., 2015). In addition, stressors

such as drought, which is considered a primary constraint to agriculture compromised the yield and quality of the cereals produced, thus, increasing the risk to global food security (Lobell and Gourdji, 2012).

Therefore, the development of cultivars with drought adaptations and high grain yield has been the focus of many plant-breeding programs. However, this is a major challenge since the loss of production caused by drought is complex and influenced by duration, intensity, phenological stage and genetic material (Araus et al., 2012). Specifically

in maize, when it occurs in pre-flowering, with high intensity and average duration of twelve days, it can reduce final grain yield by up to 60%. This yield reduction is directly related to the reduction of the number of rows per ear and to the number of grains per row. Additionally, it may be associated with the grain filling (Magalhães and Durães, 2008) due to reduction in the content, functionality and structure of starch (Thitisaksakul et al., 2012).

Several works related the drought's ability to change in the organic composition of grain in crops such as barley (Maleki-Farahani et al., 2011), maize (Masoero et al., 2013) and wheat (Singh et al., 2007). In addition, a correlation was observed between starch and protein content in grains of plants submitted to water deficit. In these plants, when the starch content is high, the protein content is lower. On the other hand, under water deficit, where the starch is reduced (Ahmadi and Baker, 2001), protein levels significantly increased (Oktem, 2008). In addition, it was verified that the levels of several nutrients can increase in grain during drought (Ge et al., 2010; Maleki-Farahani et al., 2011).

However, it is important to highlight the observations made in the 1990s by Rengel et al. (1999) and reaffirmed by Ashraf (2014) that the grain yield and the nutritional quality of the grain is influenced by soil type, nutrients availability, plant species, stress severity, genetic material and phenological stage that stress occurs. Thus, aimed with this work the changes in grain yield and in organic and mineral composition of grains in four maize genotypes with contrasting characteristics for drought tolerance submitted to severe drought in pre-flowering was evaluated.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse condition at EmbrapaMilho e Sorgo (19°28 'S, 44°15'08' 'W, 732 m altitude). Plant material consisted of four maize genotypes with contrasting characteristics for drought tolerance, two drought-tolerant (DKB390 and P30F35) and two drought-sensitive (BRS1010 and 2B710). The design was completely randomized in a factorial scheme 2 × 4, with two water conditions (field capacity and water deficit) and four genotypes (DKB390, P30F35, BRS1010 and 2B710) with four replications.

Plants were cultivated in plastic recipients with capacity of 20 L containing Typic Dystrophic Red Latosol, with fertilization carried out after chemical soil analysis. Water content in the soil was monitored daily between 9 am and 3 pm with the aid of a moisture sensor model GB Reader N1535 (Measurement Engineering, Australia) installed in the center of each pot with a screw thread at a depth of 20 cm. These sensors detect soil water tension based on electrical resistance and are coupled to digital meters. Water replenishment through irrigation was performed based on the readings obtained with the sensor, until the

field capacity occurred during the period that preceded the imposition of treatments. Water replacement calculations were performed with the aid of a spreadsheet made according to water retention curve of the soil. In parallel, all necessary cultural and phytosanitary treatments were carried out according to the demand of the maize crop.

Upon reaching the pre-flowering stage, half of each initial plant was submitted to water deficit (WD) and the other half continued to receive irrigation daily in order to maintain soil moisture near field capacity (FC), with soil water tension of -18 kPa. Drought exposure was given by daily supply of 50% of the total available water until the water tension in the soil reached a minimum of -138 kPa, which was maintained for a period of twelve days. At that moment, the leaf water potential was evaluated with the aid of a pressure pump model Scholander at midday (Ψ_{md}). After this period the irrigation was again reestablished at the field capacity level until the harvest.

At the end of the crop cycle, the ears were harvested and analysis performed at ear level, among them, the number of rows per ear and the number of grains per row by direct count, ear diameter and ear length with aid of a pachymeter and a ruler graduated, respectively. After these analyses the grains were removed and dried in a forced air circulation stove at 65°C. Further, grains were ground in a mill model Willye, showing mean particle size of less than 1 mm; this dried and ground material was used in organic and mineral analysis.

For carbohydrate extraction, 200 mg of grains was homogenized in 5 ml of 100 mM potassium phosphate buffer (pH 7.0), followed by a water bath at 40°C for 30 min. Subsequently, the centrifugation was performed at 5.000 g for 10 min and the supernatant collected. This procedure was performed twice and the supernatants combined (Zanandrea et al., 2010). This extract was used to quantify reducing sugars by the dinitrosalicylic acid method (Miller, 1959) and sucrose extracted (Van Handel, 1968).

The extraction of starch was carried out by suspension of pellet in potassium acetate buffer 200 mM and pH 4.8. Subsequently, the amyloglucosidase enzyme was added to the medium and incubated in a water bath at 40°C for two hours. After the reaction, samples were centrifuged at 5,000 g for 20 min and the supernatant was collected, which had its volume filled with potassium acetate buffer to 15 ml. The quantification of starch and sucrose was performed by the Antrona method (Dische, 1962). The standard curve for spectrophotometric determination of carbohydrates was prepared with D-glucose.

Protein percentage in the dry mass of grains was determined according to nitrogen content multiplied by the conversion factor of 6.25 (Souza and Nogueira, 2005). Determination of the percentage of fiber in acid detergent (ADF) and lignin was performed by the sequential analysis proposed by Van Soest et al. (1991). The American Association of Cereal Chemists (2001) used the method 30-

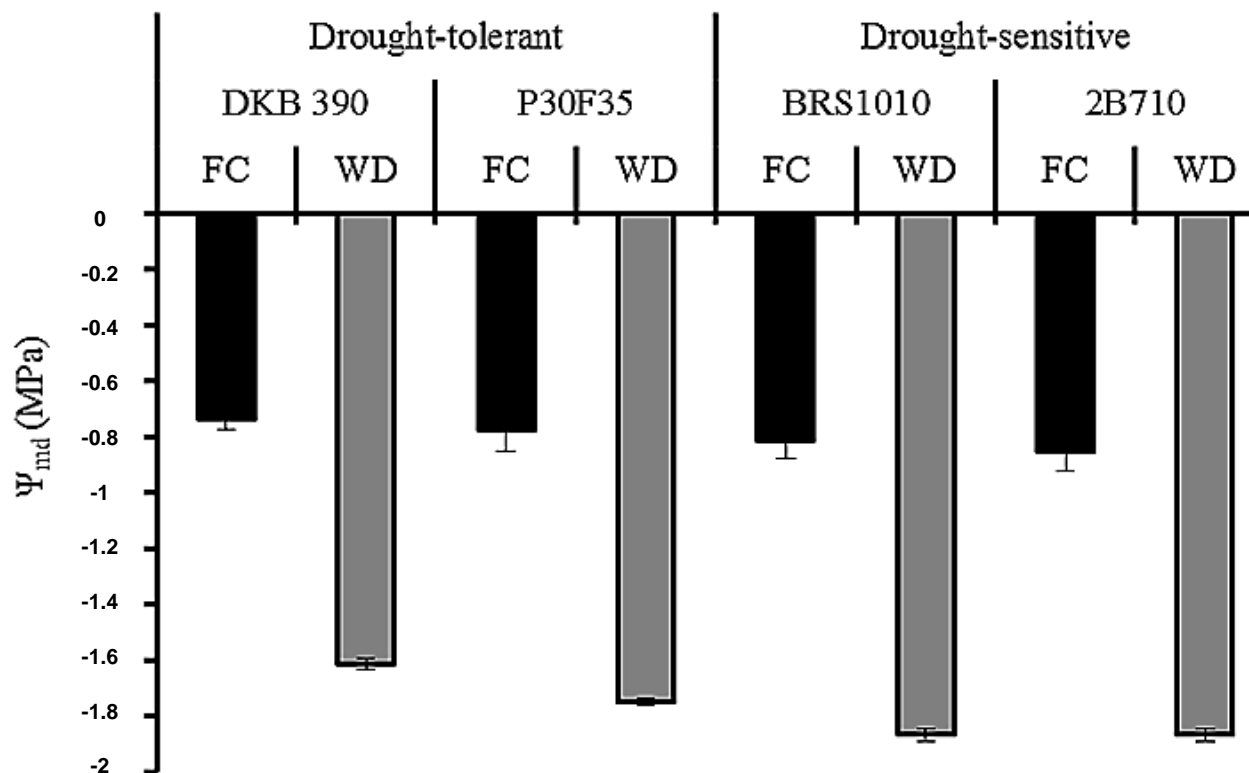


Figure 1: Leaf water potential at midday (Ψ_{md}) in four maize genotypes with contrasting characteristics for drought tolerance, cultivated under different levels of water in soil, field capacity (FC) and water deficit (WD), in pre-flowering. The bars represent the standard error of the means of four replicates.

25 to determine the percentage of lipid. The percentage of macro (N, P, K, Mg, Ca and S) and micronutrients (Zn, Fe, Mn and Cu) were determined according to the methodology described by Silva et al. (2009). After obtaining the data, preliminary statistical tests were applied to fit or not the results to analysis of variance (ANOVA). The Scott-Knott test at 5% probability was used to test all contrast between treatments.

RESULTS AND DISCUSSION

All genotypes submitted to water deficit, which exhibited soil water tension at -138 kPa, had lower leaf water potential values than their controls in field capacity (soil water tension of -18 kPa) (Figure 1). In addition, when submitted to water deficit, it can be observed that DKB390 and P30F35, drought-tolerant genotypes, showed greater leaf water potential than BRS1010 and 2B710, drought-sensitive genotypes.

Alterations occurred in leaf water potential due to water deficit which strongly influenced the components of production at level of ear, such as the number of grains per row (Figure 2A). All genotypes submitted to water deficit reduced the number of grains per row as compared to those in field capacity. This reduction was of 25 and 22%

for DKB390 and P30F35, drought-tolerant genotypes and of 35.7 and 56.5% for 2B710 and BRS1010, drought-sensitive genotypes, respectively.

Regarding the number of rows of grains (Figure 2B) under field capacity, plants of 2B710 produced highest number of rows of grains among all genotypes. In contrast, BRS1010 genotype showed the lowest values of this variable in this same condition. Water deficit did not affect the number of rows of grains in drought-tolerant genotypes. On the other hand, sensitive genotypes reduced the number of rows of grains when submitted to water deficit.

In addition, it was found that tolerant genotypes, DKD390 and P30F35, did not have ear length (Figure 2C) influenced by water deficit. In contrast, drought-sensitive genotypes, 2B710 and BRS1010, reduced ear length when compared to their controls in field capacity. It is also worth noting that BRS1010, when submitted to drought showed the lowest ear length among sensitive genotypes, reaching a reduction of 43% in ear length when compared to their control in field capacity.

Under normal soil water conditions, DKB390, P30F35 and 2B710 showed higher dry mass of grains as compared to BRS1010, a drought-sensitive genotype (Figure 2D). It was also verified that tolerant genotypes did not reduce grain yield when submitted to water deficit. In contrast,

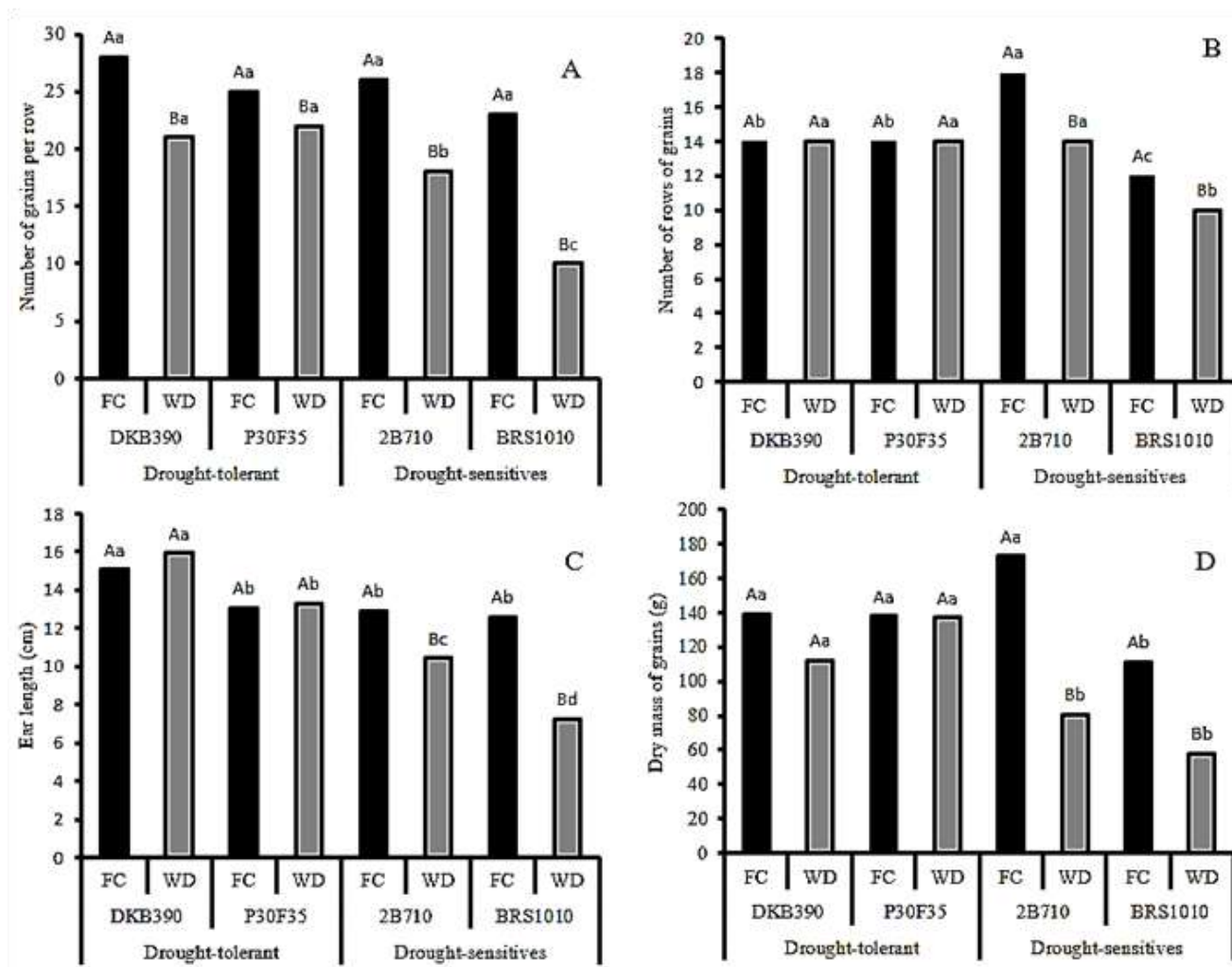


Figure 2: Number of grains per row (A), number of rows of grains (B), ear length (C) and dry mass of grains (D) in four maize genotypes with contrasting characteristics for drought tolerance, cultivated under different levels of water in soil, field capacity (FC) and water deficit (WD), in pre-flowering. Capital letters compare the each one genotype in different soil water levels; lowercase letters compare genotypes within the same soil water level by the Scott-Knott test at the 5% probability level. Means followed by the same letter are not statistically different from each other.

2B710 and BRS1010, drought-sensitive genotypes, reduced dry mass of grains in 53.25 and 47.8%, respectively, when compared to their controls in field capacity. In this way, under drought conditions, tolerant genotypes exhibited values of dry mass of grains higher than the sensitive ones.

A major cause of the reduction of 60% in maize production due to drought is the increase in the interval between male and female flowering that occurs when water deficit occurs in pre-flowering (Magalhães and Durães, 2008). This increase occurs due to the low levels of energy that plants encounter during water deficit since photosynthetic rate is reduced and limits the flow of photo assimilates to developing reproductive organs, making the plant incapable of developing these organs in synchronism (Setter and Flannigan, 2001).

In addition, low energy levels can lead to the formation of

small ears with reduced number of rows of grains and reduced number of grains per row, which contributes to lower final grain production. In addition, Lavinsky et al. (2015) working with drought-sensitive and -tolerant genotypes observed that during the water deficit, tolerant genotypes maintain greater stomatal and mesophyll conductance and consequently, higher photosynthetic rate and grain yield. Therefore, suggesting that drought-tolerant genotypes may have maintained a higher photosynthetic rate compared to sensitive ones during water deficit is important for maintaining productivity.

In addition, water deficit altered the carbohydrate standards in grains (Table 1). Reducing sugars levels remained constant in drought-tolerant genotypes, DKB390 and P30F35 and reduced in sensitive ones, 2B710 and BRS1010. It was also verified that drought-sensitive

Table 1: Organic composition of grains of four maize genotypes with contrasting characteristics for drought tolerance, cultivated under different levels of water in soil.

Parameter	Drought-tolerant				Drought-sensitive			
	DKB390		P30F35		2B710		BRS1010	
	FC	WD	FC	WD	FC	WD	FC	WD
RS*	190.6 ^{Ab}	194.7 ^{Aa}	173.0 ^{Ab}	177.4 ^{Aa}	257.5 ^{Aa}	182.8 ^{Ba}	237.1 ^{Aa}	199.8 ^{Ba}
Sucrose*	35.5 ^{Aa}	35.8 ^{Aa}	27.8 ^{Ab}	29.2 ^{Ab}	34.2 ^{Aa}	34.5 ^{Aa}	30.8 ^{Aa}	34.4 ^{Aa}
Starch*	1543.9 ^{Ab}	1185.5 ^{Bc}	1393.0 ^{Ac}	921.9 ^{Bd}	1811.5 ^{Aa}	1361.2 ^{Bb}	1780.1 ^{Aa}	1533.2 ^{Ba}
Protein (% N)	11.5 ^{Bb}	14.8 ^{Aa}	9.7 ^{Bc}	13.2 ^{Ab}	9.5 ^{Bc}	15.7 ^{Aa}	12.6 ^{Ba}	15.4 ^{Aa}
Lipids (%)	3.7 ^{Ab}	3.3 ^{Ab}	4.1 ^{Aa}	3.5 ^{Bb}	3.8 ^{Ab}	3.7 ^{Ab}	4.2 ^{Aa}	4.1 ^{Aa}
Lignin (%)	1.0 ^{ns}	0.9 ^{ns}	0.9 ^{ns}	0.9 ^{ns}	1.0 ^{ns}	1.2 ^{ns}	1.1 ^{ns}	1.6 ^{ns}
ADF (%)	6.6 ^{Aa}	5.6 ^{Bb}	6.7 ^{Aa}	4.9 ^{Bb}	5.8 ^{Ab}	6.2 ^{Aa}	6.1 ^{Ab}	6.2 ^{Aa}

Field capacity (FC) and water deficit (WD) in pre-flowering. RS- Reducing sugars; ADF: fiber in acid detergent *($\mu\text{molglucose g}^{-1} \text{DM}$). Means followed by the same letter are not statistically different from each other. Capital letters compare the each one genotype in different soil water levels; lowercase letters compare genotypes within the same soil water level by the Scott-Knott test at the 5% probability level.

genotypes showed superior values of reducing sugars in relation to the tolerant genotypes when cultivated in field capacity. On the other hand, under water deficit, these differences were not verified. Sucrose content was unaffected by water deficit. Differences were observed only among the genotypes, since P30F35 showed lower sucrose concentrations in relation to other genotypes in both water conditions.

Starch, as the main reserve in maize grains, was the carbohydrate that showed the biggest change under water deficit. Starch levels reduced in all genotypes submitted to water deficit when compared to their controls in field capacity. According to Thitisaksakul et al. (2012), moderate water deficit can reduce starch levels in grains by up to 40%. This can be due to the fact that water deficit compromises both production of photo assimilates, source of carbon skeletons for the synthesis of starch, as well as, reduces the activity of enzymes involved in starch biosynthesis in the endosperm.

Thus, it is suggested that lower starch content observed in grains of genotypes submitted to water deficit is correlated with the availability of reducing sugars as well as, with the lower activity of starch biosynthesis enzymes. Lower reducing sugars levels due to water deficit in drought-sensitive genotypes, reduced the availability of this sugar for starch biosynthesis. On the other hand, under water deficit, reducing sugars content was unaffected in tolerant genotypes, while tolerant ones also had lower starch content in the grains, which possibly occurred due to enzymatic limitation as reported by Ahmadi and Baker (2001).

It should also be noted that the ability of grains to assimilate and accumulate starch could be a genetic characteristic inherent to each genotype. In this work, when comparing the genotypes in each soil water level, it was verified that in both field capacity and water deficit, the drought-sensitive genotypes had higher starch content when compared to -tolerant genotypes, mainly in relation

to P30F35, which presented the lowest starch content among all genotypes in both water conditions.

In contrast to starch content, it was observed that water deficit positively influences protein content. Oktem (2008), with wheat cultivated at different levels of water in soil verified that when soil reaches lower water tension, protein levels increase in grains. Ge et al. (2010), Singh et al. (2008) and Oktem (2008) related that plants of maize under normal water condition showed an increase in starch content and a reduction in protein content in their grains, however, under water deficit, the inverse was verified.

In this work, it was found that, water deficit increased protein content in grains in all genotypes when compared to their controls in field capacity. In addition, in field capacity, protein levels were higher in BRS1010, followed by DKB390 and lower in P30F35 and 2B710. On the other hand, underwater deficit, P30F35 had the lowest protein content among other genotypes. In addition, protein content in grains behaves as an intrinsic factor in the genetics of each genotype and is directly related to nitrogen assimilation capacity in grain (Wang and Frei, 2011).

Lipid content was reduced with water deficit only in the drought-tolerant genotype, P30F35. In this condition, the BRS1010 genotype had lipid content higher than all other genotypes. Under normal water conditions, the highest lipid levels were observed in P30F35 and BRS1010. Regarding the lipids content, effects of water stress are varied and linked to species, genetic material and organ analyzed (Wang and Frei, 2011). In addition, most of the studies verified higher alterations in lipid composition than in the final yield of this oil (Di Caterina et al., 2007; Flagella et al., 2002). In the case of maize cultivated under water deficit, lipid content did not show variation between genotypes and only some genotypes had their content influenced by drought (Harrigan et al., 2007).

Lignin content was similar in all genotypes in both water conditions, as observed by Masoero et al. (2013). Lignin levels reduced in plants of DKB390 and P30F35 under

Table 2: Mineral composition of grains of four maize genotypes with contrasting characteristics for drought tolerance, cultivated under different levels of water in soil, field capacity (FC) and water deficit (WD) in pre-flowering.

Units	Drought-tolerant				Drought-sensitive			
	DKB390		P30F35		2B710		BRS1010	
	FC	WD	FC	WD	FC	WD	FC	WD
K (g/kg)	3.52 ^{Aa}	3.44 ^{Aa}	3.57 ^{Aa}	3.33 ^{Aa}	3.79 ^{Aa}	3.92 ^{Aa}	3.51 ^{Aa}	3.73 ^{Aa}
P (g/kg)	2.11 ^{Bb}	2.68 ^{Ab}	2.52 ^{Aa}	2.47 ^{Ac}	2.10 ^{Bb}	2.39 ^{Ac}	2.53 ^{Ba}	3.27 ^{Aa}
Mg (g/kg)	0.86 ^{Ba}	0.96 ^{Aa}	0.93 ^{Aa}	1.00 ^{Aa}	0.72 ^{Bb}	0.95 ^{Aa}	0.78 ^{Bb}	0.91 ^{Aa}
Ca (g/kg)	0.06 ^{Aa}	0.04 ^{Ba}	0.05 ^{Ab}	0.04 ^{Ba}	0.06 ^{Aa}	0.04 ^{Ba}	0.04 ^{Ac}	0.03 ^{Bb}
S (g/kg)	1.21 ^{Aa}	1.37 ^{Ab}	1.30 ^{Aa}	1.29 ^{Ab}	1.14 ^{Ba}	1.5 ^{Aa}	1.24 ^{Ba}	1.49 ^{Aa}
Zn (mg/kg)	32.82 ^{Aa}	35.62 ^{Ab}	36.88 ^{Aa}	40.11 ^{Aa}	29.02 ^{Bb}	43.67 ^{Aa}	27.71 ^{Bb}	43.33 ^{Aa}
Fe (mg/kg)	15.49 ^{Ab}	17.07 ^{Ab}	22.87 ^{Aa}	20.50 ^{Ab}	16.15 ^{Bb}	21.76 ^{Ab}	18.42 ^{Bb}	25.95 ^{Aa}
Mn (mg/kg)	4.75 ^{Ac}	5.50 ^{Ab}	7.87 ^{Aa}	8.10 ^{Aa}	6.38 ^{Bb}	8.83 ^{Aa}	6.05 ^{Bb}	8.16 ^{Aa}
Cu (mg/kg)	2.53 ^{Aa}	2.69 ^{Aa}	1.71 ^{Ab}	1.50 ^{Ac}	1.52 ^{Bb}	2.16 ^{Ab}	1.35 ^{Bb}	1.69 ^{Ac}

Means followed by the same letter are not statistically different from each other. Capital letters compare the each one genotype in different soil water levels; lowercase letters compare genotypes within the same soil water level by the Scott-Knott test at the 5% probability level.

water deficit as compared to their controls in field capacity and did not change in BRS1010 and 2B710. In drought conditions, drought-sensitive genotypes increased ADF levels when compared to tolerant ones. Contrary to what was observed in this work, Masoero et al. (2013) verified that lignin levels due to water condition do not vary.

Verifying mineral composition of grains (Table 2), no differences in potassium levels between genotypes and between water conditions were found. In turn, there was a general increase in phosphorus content in stressed genotypes when compared to their controls in field capacity. Under water deficit, phosphorus levels were higher in BRS1010, followed by P30F35 and lower in DKB390 and 2B710, which showed no differences between them. In field capacity, a similar pattern to that of water deficit was verified except for P30F35 and BRS1010 that showed similar potassium concentrations. This increase in phosphorus levels in grains, induced by drought, was also observed in barley (Farahani et al., 2011). Phosphorus levels increased due to greater allocation of this element to vegetative tissues from grains. This was suggested since phosphorus absorption during drought is reduced due to its unavailability in the soil (Rehman and Nautiyal, 2002).

It was also verified that among genotypes under water deficit, only P30F35 maintained magnesium levels equal to those observed in their control in field capacity; other genotypes increased magnesium levels under water deficit. In field capacity, drought-tolerant genotypes showed higher magnesium concentrations when compared to sensitive ones. However, under water deficit, this difference was not observed. Ge et al. (2010) also observed changes in magnesium levels in maize grains induced by water deficit. The authors in a field experiment conducted over two years observed that magnesium levels increase in maize grains as water deficit became more severe.

All genotypes, under drought, had their calcium levels

reduced in relation to their controls in field capacity. Under normal irrigation, plants of DKB390 and 2B710 did not differ from each other and presented higher calcium concentrations than plants of P30F35, especially, BRS1010. Under water deficit, the lowest calcium levels were verified in BRS1010, while other genotypes did not differ among themselves. Calcium is an essential macronutrient for plants and its transport is by mass flow, governed by transpiration (White, 2001). During severe water deficit, maize plants can reduce their transpiration by up to 70% (Lavisnky et al., 2015). Thus, it is suggested that lower calcium content observed in all genotypes, under water deficit is directly related to transpiration restriction that water deficit may have caused to genotypes. In this way, transpiration restriction could have compromised the absorption and transport of calcium to grain.

In contrast to that observed for calcium, sensitive genotypes had their sulfur and micronutrients (zinc, iron, copper and manganese) levels increased with drought and the tolerant ones, in turn, did not change levels of these nutrients. Under field capacity, all genotypes showed the same sulfur content. However, under water deficit, the drought-sensitive genotypes had higher levels of sulfur than the tolerant ones. Under field capacity, zinc content was superior in tolerant genotypes. On the other hand, under water deficit, P30F35, 2B710 and BRS1010 showed higher values of this micronutrient than DKB390.

Plants of P30F35, under field capacity, showed highest iron and manganese levels of all genotypes in the same condition. Nevertheless, under water deficit, the highest concentrations of iron were observed in BRS1010, a drought-sensitive genotype. In contrast, under water deficit, manganese levels were higher in P30F35, a drought-tolerant genotype. DKB390 showed the lowest manganese concentration among all genotypes under water deficit. Plants of DKB390, a drought-tolerant genotype, had the

highest copper content when submitted to both water conditions. On the other hand, P30F35, a tolerant genotype showed the lowest copper content among all genotypes when submitted to drought.

Farahani et al. (2011) and Ge et al. (2010) verified that grains of plants of barley submitted to water deficit showed an increase in iron, zinc and manganese nutrients as observed in this work. In addition, the authors reported that the increase in the levels of these nutrients may be related to the process of allocation of these nutrients from leaves to grains. However, Miller et al. (1994) suggested that iron, for example, has medium motility and only 20% of its content in the leaves is reallocated to the grains.

Thus, it is suggested that this increase in phosphorus, magnesium, iron, copper, zinc and manganese levels is also linked to sink strength that each grain constitutes in specific. Stressed plants showed a reduction in the number of grains per row and a greater content of these elements. In general, drought-sensitive genotypes that had the number of grains per row more affected were the ones with the highest levels of these minerals in their grains. Therefore, the increase of nutrients in the grains may be related to the number of grains formed, each grain being a specific sink.

Conclusion

Drought promoted a reduction in the number of grains per row in all genotypes of maize. However, only drought-sensitive genotypes, BRS1010 and 2B710, had a reduction in their final grain yield. Under water deficit, a reduction in the starch content and an increase in protein levels in the grains of all genotypes were observed.

Plants submitted to drought showed an increase in phosphorus, iron, magnesium, zinc, manganese and copper levels. On the other hand, water deficit reduced the calcium levels in all maize genotypes.

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